

Product information: Halo-Flipper (SC026)

Localizable Flipper-TR® membrane tension

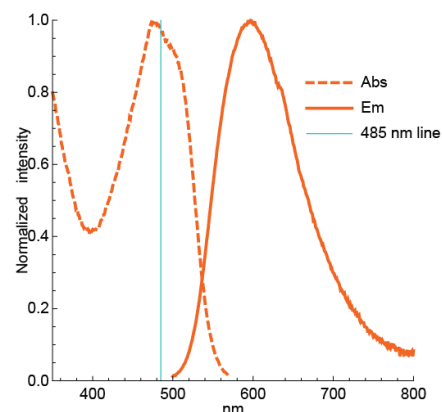
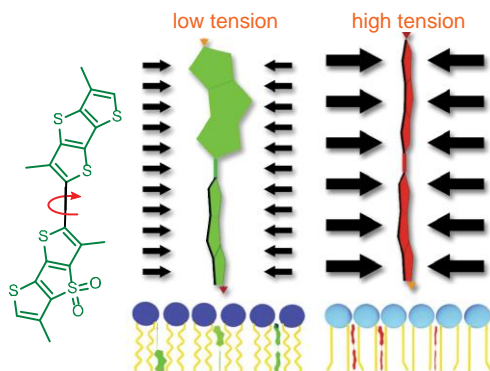
Introduction

Halo-Flipper is a fluorescent probe that specifically labels Halotag™* and reports membrane tension changes through its fluorescence lifetime changes. It contains a ChloroAlkane Halotag™* ligand as well as a tethered Flipper-TR fluorophore which senses changes of the organization of lipid bilayer membranes surrounding the Halotag™* protein. Halo-Flipper is cell permeable, spontaneously labels Halotag expressing cells and is only fluorescent when inserted in a lipid membrane. It has a broad absorption and emission spectrum, excitation can be commonly performed with a 488nm laser, while emission is collected between 575 and 625nm. It is the perfect tool to precisely localize the Flipper-TR membrane tension fluorophore within cells.

ChloroAlkane (CA) is the substrate of the self labeling tag Halotag™*. Upon reaction with a CA derivative, Halotag™* forms a covalent bond with the substrate. It allows to permanently attach a fluorescent label to any protein of interest (POI) expressed as Halotag™* fusion

Photophysical properties

λ_{Abs}	480 nm
λ_{Em}	600 nm
ϵ_{max}	$1.66 \cdot 10^4 \text{ mol}^{-1} \cdot \text{cm}^{-1}$ (DMSO)
lifetime	2.8 - 7 ns
QY	30% (AcOEt)



Absorbance and Emission of FLIPPER-TR

Labelling Protocol

Note: This protocol was optimized using HeLa cells adhering to coverslips and has been confirmed in other common cell lines. Recommendations for experimental protocols should be used as a starting point, and optimal labeling conditions for each cell type should be determined empirically.

Prepare stock solution. Dissolve the content of the vial of Halo-Flipper in 50 μL of anhydrous DMSO to make a 200 μM stock solution. This solution should be stored at -20°C or below. Do not divide the solution into small aliquots, they will decay faster and the compound is not altered by multiple freeze-thaw cycles. When stored properly, this stock solution is stable for three months.

Prepare staining solution. Dilute Halo-Flipper DMSO stock solution 1:1000 (final concentration 200 nM) in your usual cell culture medium (e.g. DMEM + 10% fetal bovine serum) and vortex briefly. Proceed quickly to step 3. Since staining efficiency can depend on the cell line, it is recommended to stain cells with 1:1000 dilution at the first attempt and then optimize the dilution factor in further experiments until an optimal staining is achieved. The usual concentration range for live cell labelling is 100-500 nM. Use only freshly made staining solution and do not use it multiple times.

Cell preparation and staining. Grow cells transiently transfected or stably expressing a Halotag™* fusion-protein on coverslips, glass bottom dish or glass bottom multi-well plates as usual. When cells have reached the desired density or expression

level of the Halotag™* fusion-protein, replace the culture medium by the **staining solution** freshly prepared under step 2 ensuring that all the cells are covered with the solution. Place the cells in the incubator at 37°C in a humidified atmosphere containing 5% CO₂ for min 30 minutes, although 1h is recommended. It is recommended to wash the cells with fresh culture medium to remove the excess of unbound Halo-Flipper.

FLIM imaging. Cells are imaged with standard FLIM microscopes using a 485 or 488 nm pulsed laser for excitation and collecting photons through a 600/50 nm bandpass filter. We recommend optimizing the labeling procedure as well as the image acquisition settings to minimize photodamage induced by the 488nm excitation light on live samples. To extract lifetime information, the photon histograms from ROI or single pixels (accumulate sufficient counts to ensure good statistics) are fitted with a double-exponential, and two decay times, τ_1 and τ_2 are extracted. The longest lifetime with the higher fit amplitude τ_1 is used to report membrane tension and varies between 2.8 and 7.0 ns. Longer lifetime means more tension in the membrane. τ_2 with a smaller value (between 0.5 and 2 ns) and a small fit amplitude is less suited to study membrane tension. The lifetime can be correlated to absolute membrane tension using the calibration procedure given in Reference 1.

Important notes:

- Membrane tension measurements can only be performed by FLIM microscopy, fluorescence intensity or wavelength is not reliably reporting on membrane tension.
- Systems where the membrane lipid composition changes over time may also induce a change of Halo-Flipper lifetime.
- FLIM imaging is an advanced microscopy technique requiring a commercial or custom built FLIM microscopy system with the adequate excitation lasers, photon counting systems and emission filters. Customers are advised to consult their instrument responsible person or contact the microscope manufacturer to ensure that their system is able to image Halo-Flipper fluorescence and lifetime.

References:

- 1) Colom A, *et al*: A fluorescent membrane tension probe. *Nat Chem*, 2018, **10**:1118–1125 ().
- 2) Straková K., *et al*: HaloFlippers: A General Tool for the Fluorescence Imaging of Precisely Localized Membrane Tension Changes in Living Cells, *ACS Cent. Sci.* 2020, **6**, 8, 1376–1385.

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